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Update on Development

Blazing New Trails[1]

Pollen Tube Guidance in Flowering Plants

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Higher plants minimize their exposure to pollen from diverse species by precise regulation of flowering time, development of floral organs, and interactions with specific pollinators. These measures do not guarantee success, however, because of the vast quantities of pollen in the environment. Consequently, to ensure that only the appropriate sperm are delivered to the eggs, many plants have evolved elaborate signaling pathways that monitor the pollen as it grows into the interior of the pistil. Signals are exchanged at every step along this pathway, beginning with the recognition of pollen by cells on the pistil surface and continuing as the pollen grains extend polarized projections (pollen tubes) that invade the pistil and migrate to the eggs. As the pollen tubes race toward their targets, these signals guide the deposition of new membrane and cell wall components and the transportation of sperm and other cellular contents to the tip of the growing tube. Whether the signals that direct pollen tube growth come from female tissues or from the pollen tube itself remains unknown; successful navigation most likely depends on the interplay of both male and female signals. These signals could be structural or chemical in nature, and probably comprise a combination of positive cues that guide pollen tubes to the eggs and negative signals that repel other tubes. Recent investigations into pollen tube guidance have brought the answers to some of these questions within reach.

OVERVIEW OF PLANT REPRODUCTION

Pollen tubes were first discovered in 1824 by Italian astronomer Giovanni Batista Amici (1847), and since that time, the details of pollen tube growth from stigma to ovule have been documented in a large number of plant families (Fig. 1A). Although there is significant variation in the detail, pollination can be divided into characteristic stages that include (a) pollen hydration and germination, (b) growth of the pollen tube through the stigma, style, and ovary, (c) pollen tube guidance to the ovule micropyle, and (d) delivery of the sperm to the embryo sac (Fig. 1, A and B). The time required for these events varies considerably between species: *Brassica* require only a few hours for pollination, but pines require many months.

Early signaling events lead to pollen hydration on the stigma, and this step can be highly regulated; dry stigmas typically transfer water and nutrients only to pollen from closely related species. On the other hand, wet stigmas are less selective, secreting a nutrient-rich exudate that promotes pollen hydration in a somewhat indiscriminate manner. After hydration, the pollen grains germinate and form tubes that invade or grow between the stigma cells and subsequently migrate into the pistil. In the style the pollen tubes grow through an extracellular matrix that either lines a hollow channel or is secreted between style cells that make up a solid transmitting tract. The composition of this complex matrix includes lipids, proteins, carbohydrates, and small molecules. It is believed that these components provide the signals that are critical for directing and supporting pollen tube growth. Once the pollen tubes have migrated into the ovary, they abandon the nutrient-rich environment of the style, enter the ovary locule, and grow toward the ovules. In some species the route to the ovules is marked by a trail of secreted material, but more often, the tubes travel in a relatively dry environment along the surface of the cells that physically support the ovule. Upon arrival at the micropyle, the tip of the pollen tube enters the embryo sac via a synergid cell and bursts, releasing the sperm cells, which migrate to the egg and central cell for fertilization.

POLLEN TUBE ANATOMY AND SIGNALS THAT ORIENT TUBE GROWTH

Growing pollen tubes transport all of their cellular contents toward the tip of the tube, most likely by using networks of actin and myosin (Mascarenhas, 1993). Secretory vesicles converge at the tip while other organelles are distributed in the distal regions. This polarized organization of the tube cell is reminiscent of that found in the buds of growing yeast cells. The recent localization of small

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Abbreviations: SLG, S-locus glycoprotein; SRK, S-locus receptor kinase; TTS, transmitting tissue-specific.

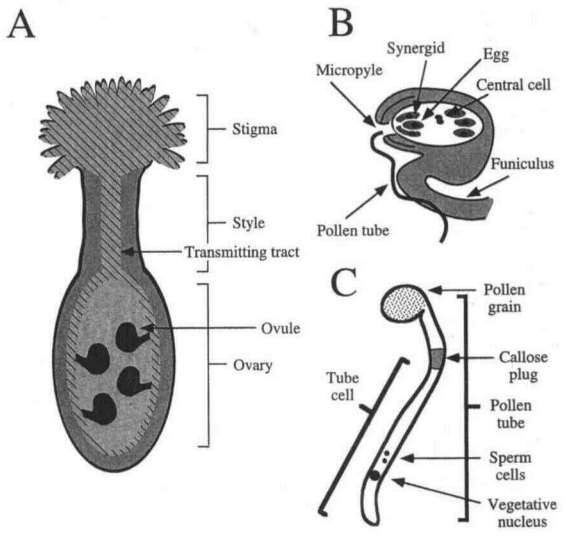

Figure 1. Reproductive structures in flowering plants. A, The pistil contains a stigma, style, and basally located ovary that encases the ovules. Pollen tubes travel from the stigma to the ovary through the transmitting tract. B, The ovule contains the female gametophyte, composed of an egg, a 2*n* central cell, two synergids that reside next to the egg, and three antipodal cells that are opposite the micropyle. Pollen tubes typically travel up the funiculus and enter the ovule through the micropyle. There, they penetrate the synergid cell and deliver two sperm that eventually fuse with the egg and central cell, forming the zygote and 3*n* endosperm, respectively. C, Pollen grains, the male gametophytes, carry the two sperm and the vegetative nucleus to the female gametophyte by forming a pollen tube. Plugs of callose separate older parts of the tube from the tube cell at the growing tip.

GTP-binding proteins, which potentially share common functions, to the tips of pollen tubes (Lin et al., 1996) and the surface of yeast buds (Ziman et al., 1993) suggests that the similarities extend to the molecular level. Along with numerous organelles, each pollen tube cell contains a nucleus that actively transcribes messages needed for growth and two sperm cells with condensed and inactive nuclei that arise from a mitotic division of the generative cell (Fig. 1C). The volume of the tube cell remains relatively constant as "plugs" of callose are periodically deposited; these plugs completely seal off the newer portion of the tube, and fertilization can proceed even if the pollen grain and older sections are excised (Jauh and Lord, 1995).

Ca^{2+} has long been known to play a key role in the growth of pollen tubes. Recent studies indicate that gradients of Ca^{2+} in pollen tubes are critical not only for pollen tube growth, but also for guidance. Studies of pollen from several species have demonstrated that Ca^{2+} levels are high at the tip of the pollen tube and rapidly dissipate through the distal portions. Oscillations in pollen tube growth correlate with fluctuations in this Ca^{2+} gradient; buffers that eliminate the gradient also arrest pollen tube growth (Pierson et al., 1994, 1996). Additional support for the ion fluxes as regulators of guidance comes from experimental manipulation of the Ca^{2+} gradient. Disrupting this gradient, either by iontophoretic microinjection or by incubation with Ca^{2+} channel blockers, can change the direction of tube growth, and the application of weak electric fields elicits a specific reorientation toward the cathode (Malho et al., 1994). Whether Ca^{2+} by itself guides pollen tube growth in vivo remains to be demonstrated, but its profound effects in vitro present intriguing possibilities for the regulation of guidance through the distribution of Ca^{2+} channel activity.

POLLEN-SPECIFIC GENES AND POLLEN TUBE GUIDANCE

Genes expressed by the haploid, vegetative pollen nucleus may significantly contribute to the growth of pollen tubes, enabling them to compete effectively with each other. Expression studies show that approximately 10 to 20% of pollen cDNAs are pollen-specific, and transcription of these genes occurs at two key points in pollen development: either immediately after meiosis or after the mitotic division that gives rise to the generative and vegetative cells (Mascarenhas, 1993). Thus, growing pollen tubes contain significant levels of pollen-specific messages, many of which could respond to guidance cues from the pistil. It is interesting that recent evidence indicates that these pollen-specific mRNAs can be localized to distinct regions of the pollen cytoplasm, concentrated either in the pollen grain or in the tip of the growing tube (Torres et al., 1995). This unequal distribution of pollen messages may contribute to the localized concentrations of specific proteins and may perhaps control guidance by organizing pollen tube polarity.

SIGNALS FROM THE PISTIL

Although analysis of pollen tube growth in vitro has greatly facilitated investigations of potential signaling molecules, the rate of pollen tube growth in defined medium rarely approaches that observed in the pistil. Thus, pollen tubes most likely obtain both nutritive support and guidance cues from female tissues. Indeed, as pistils develop they acquire the ability to direct the precise growth of pollen tubes. Pollination of immature Arabidopsis pistils leads to a dramatic decrease in the rate of pollen tube growth, as well as defects in pollen tube guidance, with tubes invading the stigma and growing toward the base of the pistil, but subsequently bypassing the transmitting tract and growing in the cortex of the ovary (Kandasamy et al., 1994). Pistils also acquire the ability to discriminate among pollen grains as they mature—the pollen rejection typically observed in self-incompatible species can be overcome by pollinating immature pistils. Likewise, mature Arabidopsis pistils prevent *Brassica oleracea* pollen tubes from approaching the ovules, whereas immature pistils are markedly less selective (Kandasamy et al., 1994). In cases where pollen tube growth to the ovules requires several months (such as in orchids), pistil development is coordinated with pollination and is mediated by long-range signaling via the hormones ethylene and auxin (Zhang and O'Neill, 1993). As described below, many factors have been implicated in pollen tube growth and guidance, and in

many cases these play a specific role in the stigma, style, or ovary.

COMMUNICATION BETWEEN POLLEN AND THE STIGMA

The stigma is an important barrier to both foreign pollen and pathogens, and it also provides factors that stimulate the growth of appropriate pollen tubes. Recent studies have dissected the role of the stigma by genetically ablating stigmatic tissue through the specific expression of cytotoxins. This treatment caused the complete elimination of pollen germination in *B. oleracea*, which normally has a dry stigma (Kandasamy et al., 1993). Curiously, a similar alteration in a close relative, *Arabidopsis thaliana*, did not affect the germination or invasion of wild-type pollen tubes, even though the stigma cells were greatly reduced in size and metabolic activity (Thorsness et al., 1993). Stigmatic ablation in *Nicotiana*, which normally has a wet stigma surface, did not inhibit pollen germination, but instead impaired penetration of the pollen tubes into the style, a defect that could be rescued by wild-type extracts (Goldman et al., 1994). The different results obtained in these experiments may reflect the differences in promoter strength or specificity; alternatively, they might derive from fundamental differences in stigma function.

Although severe alterations such as those described above convincingly demonstrate a role for the stigma, identification of specific signaling molecules has proven to be more difficult. Considerable progress has been made from recent investigations of the maize and petunia mutants that are deficient in flavonol biosynthesis. These mutants harbor severe defects in pollen germination and pollen tube growth, both in vitro and in vivo; these phenotypes can be rescued by adding micromolar quantities of the flavonol kaempferol (Mo et al., 1992). The mutants are self-sterile, indicating that flavonols can be supplied by either male or female tissue. Furthermore, the temporal regulation of kaempferol synthesis in the stigma defines a developmental window for successful pollination (Pollak et al., 1993). Although the requirement for flavonols has been conserved from maize to petunia, it is not universal because a lack of flavonols in Arabidopsis does not affect fertility (Burbulis et al., 1996).

Molecules on the surface of pollen also play an important role in signaling, especially in species with dry stigma surfaces. For members of the crucifer family, the initial recognition of a pollen grain is mediated by the pollen coat, an extracellular layer of proteins, carbohydrates, lipids, and small molecules deposited on the pollen surface by the anther tapetal cells (Elleman et al., 1992). Mutations in Arabidopsis that disrupt this pollen coating lead to a rejection response by the stigma: papillae cells that contact the aberrant pollen produce callose, which isolates the pollen grain and presumably prevents its hydration (Preuss et al., 1993). These mutations affect the synthesis of long-chain lipids and often the abundance of other pollen coat components, defining an intriguing role for long-chain lipids in pollen-pistil signaling (Preuss et al., 1993; Hülskamp et al., 1995a).

Self-incompatible plants incorporate complex recognition systems, active in both male and female tissues, which enable them to discriminate against pollen from genetically similar individuals. In *Brassica* and *Papaver*, this mechanism arrests the growth of incompatible pollen tubes on the stigma well before they consume valuable pistil resources (Nasrallah et al., 1994). Self-incompatibility in *Brassica* depends upon a highly polymorphic locus that encodes two stigma proteins, the SRK and the SLG; mutations in either the SRK or SLG genes can result in self-compatibility. Pollen molecules that interact with these stigma proteins have long evaded detection, but recent studies implicate two interesting components: (a) an anther-specific transcript (SLA) that is encoded by the S-locus of plants with an S2 self-incompatibility haplotype but is absent from self-compatible species (Boyes and Nasrallah, 1995), and (b) proteins in the pollen coat that bind to SLG with a high degree of specificity (Doughty et al., 1993). Self-incompatible *Papaver* species also block pollen tube growth at the stigma, but the molecules required for this response differ from those in the *Brassica* system. A simple in vitro pollen tube growth assay has allowed identification and cloning of stigma and pollen proteins that function in self-incompatible *Papaver*. It is interesting that at least one of the pollen components is phosphorylated during the self-incompatibility response, a modification that relies on a Ca^{2+}-calmodulin signaling pathway (Hearn et al., 1996; Rudd et al., 1996).

Thus, both self-compatible and -incompatible systems are providing important insight into the regulation of pollen-stigma interactions and, as described above, are uncovering a large number of interesting molecules. Whether these molecules regulate pollination in a limited number of species or play a universal role is one of the larger questions in this field. The mechanisms that regulate transduction of these early signaling events and the subsequent migration of pollen tubes into the style remain unresolved.

POLLEN TUBE GROWTH THROUGH THE STYLE

Within the style, pollen tubes migrate in a nutrient-rich matrix secreted by the female cells, traveling distances ranging from a few millimeters to several centimeters. Whether pollen tubes actively communicate with pistil cells in the style or passively follow a predetermined path is presently unclear. Support for the latter possibility comes from experiments in which latex beads, comparable in diameter to pollen tube tips, were applied to the surface of the styles from three different species, following excision of the stigma (Sanders and Lord, 1989). Regardless of style orientation, the transport of the beads toward the ovary mimicked the growth of pollen tubes, surprisingly following the appropriate pathway and migrating at comparable rates. These observations suggest that pollen tube traffic in the style might be controlled solely by the female tissues of the flower through a matrix-driven mechanism that guides the tubes from the stigma to the micropyle.

What are the matrix components that mediate pollen tube migration through the style? Recent studies in tobacco

implicate two TTS proteins in providing both nutritional support and guidance cues (Cheung et al., 1995; Wu et al., 1995). In vitro pollen tubes remove the arabinogalactan moieties from the TTS proteins, suggesting that these sugar residues serve as important nutrients. It is interesting that the style contains a gradient of TTS protein glycosylation with the highest levels at the base; this gradient may guide pollen tubes, providing nutritional support after they have expended their own resources. In support of this idea, transgenic plants with reduced levels of TTS proteins are impaired in their ability to support pollen tube growth.

In members of the self-incompatible *Solanaceae*, components in the style matrix can also arrest the growth of pollen tubes. In these species the S-locus encodes a style-specific RNase that is necessary and sufficient for pollen tube rejection (Lee et al., 1994; Murfett et al., 1994). Although the molecular mechanism is unknown, mutant S alleles that lack RNase activity no longer reject incompatible pollen, indicating the S-RNase plays a critical role (Huang et al., 1994). Analysis of additional mutants promises to define the structural and functional features that provide S-RNase specificity.

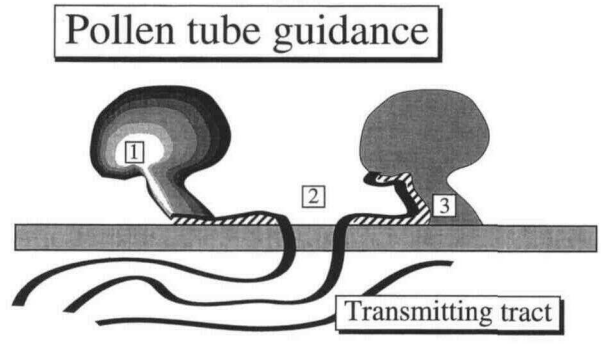

Figure 2. Proposed interactions in pollen tube guidance. Pollen tubes (thick lines) exit the transmitting tract to approach individual ovules. Chemoattractant molecules may diffuse from the micropyle to lure nearby pollen tubes (1), whereas other factors may act to prevent pollen tubes from approaching ovules that have already engaged a tube (2). Pollen tubes interact with ovary and ovule cells through tight, adhesive contacts (diagonal lines, [3]) that potentially mediate communication. The precise targeting of pollen tubes to the ovules may rely upon a combination of these signals.

TARGETING POLLEN TUBES TO THE OVULES

After leaving the style, the pollen tubes of many species no longer have access to a predetermined path of exudate, but instead navigate across the exposed surfaces of the pistil cells until they reach an ovule. Recent studies indicate that essential signals for pollen tube guidance most likely come from the ovules themselves. Pollen tubes often migrate to the first available ovule, emerging from a nearby site in the transmitting tract (Hülskamp et al., 1995b). Moreover, mutants with defective ovules show aberrant guidance, with pollen tubes meandering through the ovary (Hülskamp et al., 1995b; Sniezko and Winiarczyk, 1995). The most severe guidance defects occur when the ovules lack an embryo sac; in Arabidopsis pollen tubes not only wander past these defective ovules, but also emerge from the transmitting tract at random locations (Hülskamp et al., 1995b). These observations indicate that pollen tube guidance out of the transmitting tract and through the ovary requires directional cues. Furthermore, even without the appropriate navigational signals, pollen tubes have adequate nutritional support to travel great distances.

What types of signaling events occur at this late stage in pollen tube growth? Chemoattractants most likely direct pollen tubes to the ovules, but other signals may play an important role (Fig. 2). In particular, receptors that mediate the adhesion of pollen tubes to the pistil cells may be critical, with pollen tube growth involving weak binding interactions at the migratory tip of the tube and tight adhesion to the female tissues in the stationary distal zone (Lord et al., 1996). Because only one tube approaches each ovule (Wilhelmi and Preuss, 1996), pollen tubes most likely repel each other on any individual funiculus. This mechanism results in an effective block to polyspermy, which is analogous to that seen in many animal systems.

Although the developmental mutants described above clearly point to ovules as a source of guidance cues, precise definition of signaling components will require either in vitro assays that reproduce guidance with high fidelity or the isolation of mutants that selectively eliminate signaling molecules. The recent identification of an Arabidopsis mutant that disrupts the guidance of pollen tubes to the ovules without altering pistil structures or the rate of tube growth represents a significant advance (Wilhelmi and Preuss, 1996). In this mutant adhesion between pollen tubes and ovule cells is defective. Although random growth of the pollen tubes takes them within proximity of the micropyle, they suffer from a severe targeting defect and are unable to enter the embryo sac. This mutant, defective in two functionally redundant genes that are required for pollen-pistil interactions, *POP2* and *POP3*, is self-sterile, indicating that both male and female tissues play a key signaling role. Identifying the molecular basis of the *pop2*, *pop3* defect will prove valuable in defining late signaling components.

DELIVERY OF SPERM TO THE FEMALE GAMETOPHYTE

After a pollen tube arrives at the micropyle, it ruptures and releases two sperm, one that fertilizes the egg cell and a second that fertilizes the central cell (Fig. 1). Because these events occur rapidly and involve extremely small cells, the identification of guidance signals has proven difficult; yet, the precise growth of pollen tubes into the micropyle and the proficient fusion of sperm cells with their targets suggest that chemoattraction is important. Attractants might come from a synergid cell in the female gametophyte that, in many cases, begins to degenerate just before the pollen tube arrives (Fig. 1). Actin filaments from this degenerating cell may participate in propelling the sperm cells, which lack flagella, to their targets (Huang and

Russell, 1994). Large portions of the walls that encase the gametes degenerate immediately after the pollen tube bursts, allowing rapid cell fusion. Recently, a powerful in vitro system that recapitulates these fusion events has been developed; this technology will undoubtedly be instrumental in defining molecules that regulate fertilization (Faure et al., 1994).

CONCLUDING REMARKS

In animal systems cell migration governs a wide array of developmental processes, including the invagination that accompanies early embryogenesis, the formation of an extensive neural network, the migration of immune cells to their site of action, and the pathogenic migration of metastatic tumors. Pollen tubes represent a special case of cell migration: they communicate with pistil cells through thick, external walls and, unlike other plant cells, have a unique ability to move from their sites of origin and grow through unrelated tissues. The ability to obtain these migratory cells in abundance, to culture them in vitro, and to analyze their function at the genetic level makes this an attractive system. Whether plant and animal cells depend on the same types of molecules for their migration remains to be seen, but recent analysis of pollen tube guidance provides evidence of a tantalizing similarity to axon growth, requiring a blend of cell adhesion, repulsion, and chemoattractive signals.

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